Involvement of reflex urethral closure mechanisms in urethral resistance under momentary stress condition induced by electrical stimulation of rat abdomen

Izumi Kamo and Tadatoshi Hashimoto

Pharmaceutical Research Division, Takeda Pharmaceutical Company, Limited, Osaka, Japan

Submitted 27 November 2006; accepted in final form 27 June 2007

Kamo I, Hashimoto T. Involvement of reflex urethral closure mechanisms in urethral resistance under momentary stress condition induced by electrical stimulation of rat abdomen. Am J Physiol Renal Physiol 293: F920–F926, 2007. First published July 11, 2007; doi:10.1152/ajprenal.00466.2006.—A novel method for evaluating the urethral resistance during abrupt elevation of abdominal pressure was developed in spinalized female rats under urethane anesthesia. Electrical stimulation of abdominal muscles for 1 s induced increases in both the intra-abdominal and the intravesical pressure in a stimulus-dependent manner, and the bladder response was almost lost when the abdomen was opened. The lowest intravesical pressure during electrical stimulation that induced fluid leakage from the urethral orifice (leak point pressure) and the maximal intravesical pressure without urine leakage below the leak point pressure were evaluated as the indexes of urethral resistance. Lower urethral resistance was obtained in the rats whose pelvic nerves or somatic nerves containing pudendal nerves and nerves to iliococcygeus/pubococcygeus muscles were transected bilaterally. In contrast, transection of bilateral hypogastric nerves showed smaller effects. Duloxetine, a drug for stress urinary incontinence, enlarged the reflex urethral closing contractions that were induced by an increase in intravesical pressure and measured using a microtip transducer catheter in the middle urethra. This drug also increased the urethral resistance (leak point pressure), whereas it did not show any effect in the rats whose pelvic nerves were bilaterally transected, showing that the augmentation of the reflex urethral closure by the drug resulted in the elevation of the urethral resistance. From these findings, it was concluded that during momentary elevation of abdominal pressure, the reflex urethral closure mechanisms via bladder-spinal cord-urethral sphincter and pelvic floor muscles greatly contribute to the increase in the urethral resistance to prevent the urinary incontinence.

stress urinary incontinence; leak point pressure; reflex urethral closing responses; duloxetine

STRESS URINARY INCONTINENCE (SUI) is defined as the involuntary loss of urine during increments of abdominal pressure (stress condition) such as sneezing and coughing in the absence of bladder contractions (5, 22). This disorder is very common in women over middle age and generally occurs as a result of defects in the various passive and reflex mechanisms that maintain urethral closure in the presence of elevated abdominal pressure (1, 4, 23). Recently, the nerve-mediated reflex urethral closure mechanisms have been considered to contribute to the urethral resistance during events causing elevation of intravesical pressure (Pves) in rats (14). Electrophysiological studies also have provided evidence indicating functions of the glutamate-mediated bladder-to-urethral reflexes in rats (6, 16, 17). With the accumulation of findings of active urethral closure mechanisms, pharmacotherapy by drugs that augment the reflex urethral closing mechanisms is accepted (4, 10, 23). In fact, duloxetine, a 5-hydroxytryptamine and norepinephrine reuptake inhibitor, can increase the electromyographic activity of the external urethral sphincter muscle in cats (25) and is clinically effective as the drug for SUI (4, 23). However, a lack of variety of validated methods for measuring the urethral resistance during momentary elevation of abdominal pressure seems to limit the number of studies for elucidation of physiopathology of SUI to develop novel treatments for SUI.

Because SUI occurs when Pves exceeds the urethral resistance, and the lowest Pves value that causes urinary incontinence is almost the same as total urethral resistance, Pves is used clinically to represent the urethral resistance as a leak point pressure (LPP) (19). Recently, several kinds of methods for measuring the LPP have been developed in animal experiments (3, 7, 8, 14, 15, 24); however, these methods are not accompanied by an abrupt increment of abdominal pressure, except for the sneeze LPP (7, 15). In this study, a unique method for evaluating the urethral resistance during momentary elevation of abdominal pressure by electrical stimulation of the abdominal muscles was developed, the role of nerve-mediated urethral closing reflexes in the urethral resistance under momentary stress condition was clarified, and the effects of duloxetine on the reflex urethral closure and the urethral resistance were studied.

METHODS

Animals. Adult female rats of Sprague-Dawley strain weighing 193.5–324.9 g (CLEA Japan, Tokyo, Japan) were studied using experimental protocols approved by Takeda’s Experimental Animal Care and Use Committee.

LPP measurement using electrical stimulation of abdominal muscle. After rats were anesthetized with halothane (Takeda, Osaka, Japan) inhalation, the spinal cord was transected at the T8–T9 level after laminectomy. A sterile sponge (Astellas, Tokyo, Japan) was placed between the cut ends of the spinal cord, and the overlying muscle and skin were closed with glue. Under this condition, it has been reported that supraspinal reflex voiding is eliminated, whereas urethral reflexes induced by bladder distention, which are predominantly organized in the lumbosacral spinal cord, are preserved. The urinary bladder was exposed through an abdominal incision, and a polyethylene catheter with a fire-flared tip (PE-90; Clay Adams, Parsippany, NJ) was inserted into the bladder from the dome and secured with ligature for filling and pressure recording. The abdomen was then closed with sutures. Two sites of abdominal skin near the right and left tips of the 11th–13th costae were cut to expose abdominal muscles (abdominal oblique muscles) for insertion of the stimulus needle electrodes (Fig. 1A).
Reflex urethral closing responses induced by an increase in $P_{ves}$. The reflex urethral closing responses were induced according to the method of Kamo et al. (14) with some modifications. Under halothane anesthesia, spinal cord transection and bladder catheter insertion were performed. The bladder neck was ligated with suture to prevent fluid leakage from the bladder into the urethra. After the surgery, halothane anesthesia was switched to urethane anesthesia (1.2 g/kg ip). A 3.5-Fr-size nylon catheter with a side-mounted microtip transducer catheter located 1 mm from the catheter tip (SPR-524; Millar Instruments, Houston, TX) was inserted into the middle urethra 12.5–15 mm from the urethral orifice with the side-mounted sensor facing the inner urethral surface in the 3 o’clock position. The length of the catheter inserted into the urethra from the urethral orifice and its orientation were monitored to confirm that the position of the transducer was not moved throughout the experiments. $P_{ves}$ was controlled by connecting a bladder catheter to a saline reservoir and pressure transducer via three-way stopcocks. The reservoir was mounted on a metered vertical pole for controlled height adjustment. $P_{ves}$ was abruptly increased by elevating the reservoir and maintaining $P_{ves}$ for 30 s at the pressure of 25 or 50 cmH2O (see Fig. 3A). Between each pressure rise, the reservoir was returned to 0 cmH2O. A microtip transducer catheter was connected to an amplifier, and urethral responses were digitally recorded at a sampling rate of 500 Hz using data-acquisition software on a computer system equipped with an analog-to-digital converter. Smoothing was performed on the acquired data (smoothing factor: 500, mean value), and the baseline value before $P_{ves}$ elevation and the maximal value during increments of $P_{ves}$ were evaluated. Since the first urethral response measured tended to be smaller than the second response but seemed to become stable thereafter, measurements at $P_{ves}$ of 25 and 50 cmH2O were repeated three times each, and the last two measurements were averaged to calculate the urethral response.

Drugs. Duloxetine purchased from Kemprotec (Middlesbrough, UK) was dissolved in saline solution and administered intravenously at a volume of 1 ml/kg. Each rat received duloxetine or its vehicle only once.

Statistical analysis. Data are means ± SE. Data were analyzed with Dunnett’s test, Student’s t-test, or paired t-test, and P values <0.05 were considered to be significant.

RESULTS

Changes in intra-abdominal pressure, $P_{ves}$, and urethral pressure during electrical stimulation of abdominal muscles.

The electrical stimulation of abdominal muscles (abdominal oblique muscles) with rectangular electric pulses of 2.5, 3.0, 3.5, and 4.0 V (50-Hz, 0.5-ms width pulse trains lasting 1 s) induced increases in both the intra-abdominal pressure and $P_{ves}$ in a stimulus-dependent manner (Fig. 1B). The increment of intra-abdominal pressure was 8.9 ± 1.2, 16.5 ± 1.9, 26.1 ± 2.5, and 36.6 ± 4.7 cmH2O (2.5, 3.0, 3.5, and 4.0 V, respectively; n = 3), and elevation of $P_{ves}$ was 9.6 ± 1.6, 14.5 ± 0.7, 23.1 ± 0.3, and 32.1 ± 1.8 cmH2O (2.5, 3.0, 3.5, and 4.0 V, respectively; n = 3), showing that the magnitude of increment was similar between the intra-abdominal pressure and $P_{ves}$.

The effects of opening the abdomen on the changes in $P_{ves}$ and urethral pressure were examined in five rats. The blader neck was ligated to measure the $P_{ves}$ and the urethral pressure separately, and the urethral orifice was also ligated to fill saline into the urethra for measurement of inner pressure change. The abdominal muscles were electrically stimulated, with the stimulus intensity inducing the elevation of $P_{ves}$ by ~40 cmH2O, and then the abdomen was opened and the bladder was kept from surrounding tissues such as intestines and abdominal muscles to avoid compression of the bladder by these tissues.
Calculated in each rat. The bladder neck and the urethral orifice were ligated, and the urethra was filled with saline. The abdominal muscles were electrically stimulated 3 times, and the averages in 3 trials before and after the abdomen was opened under urethane anesthesia were calculated in each rat.

**Table 1. Increments of Pves and urethral pressure during electrical stimulation of abdominal muscles**

<table>
<thead>
<tr>
<th></th>
<th>Abdomen Closed</th>
<th>Abdomen Opened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pves, cmH2O</td>
<td>42.1±1.4</td>
<td>2.0±0.8</td>
</tr>
<tr>
<td>Urethral pressure, cmH2O</td>
<td>19.1±2.5</td>
<td>0.3±0.9</td>
</tr>
</tbody>
</table>

Data are means ± SE of intravesical pressure (Pves) and urethral pressure in 5 female spinalized rats. The bladder neck and the urethral orifice were ligated, and the urethra was filled with saline. The abdominal muscles were electrically stimulated 3 times, and the averages in 3 trials before and after the abdomen was opened under urethane anesthesia were calculated in each rat.

The electrical stimulation-induced Pves elevation was almost lost after the abdomen was opened at the same stimulus intensities (Table 1), indicating that the Pves elevation during electrical stimulation was caused by the increment of abdominal pressure. The increment of urethral pressures by ~20 cmH2O was also observed during electrical stimulation of the abdomen, and this elevation was completely lost by opening the abdomen (Table 1), indicating that the elevation of abdominal pressure induced the observed increment of the urethral pressure.

**Measurement of urethral resistance during electrical stimulation of abdominal muscles.** Although the urethral pressure measured in the urethra filled with saline reflected the urethral resistance, it was not the accurate total urethral resistance in the empty urethra, which shows the different responses depending on the portion of this organ (14); therefore, the LPP was evaluated to indicate the total urethral resistance. The electrical stimulation of rat abdominal muscles with gradual elevation of stimulus voltage again increased Pves in a stimulus-dependent manner (Fig. 2), and the measured pressure value peaked at 0.3–1 s after the stimulation started. During stimulation, 22 of 28 rats showed fluid leakage from the urethral orifice; the LPP value (Fig. 2) of these 22 rats was 49.3 ± 1.5 cmH2O. If the LPP value was assumed to be greater than the maximal Pves value during electrical stimulations in the 6 rats without fluid leakage, the average LPP value in 28 rats was >53.0 ± 2.5 cmH2O (Table 2). The MTP (Fig. 2, asterisk) was also evaluated to show the urethral resistance. This parameter indicates that below this value, no urinary leakage is observed. The average MTP value in the 22 rats with urinary leakage was 47.4 ± 1.5 cmH2O, and the assumed MTP value in all 28 rats was close to the assumed LPP value (Table 2).

**Table 2. Effects of transection of nerves innervating bladder, urethra, and pelvic floor muscles on urethral resistance during electrical stimulation of the abdomen**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leak/Tested</th>
<th>MTP (Without Leak), cmH2O</th>
<th>LPP (With Leak), cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>22/28</td>
<td>&gt;51.4±2.6</td>
<td>&gt;53.0±2.5</td>
</tr>
<tr>
<td>Pelvic</td>
<td>10/10</td>
<td>36.5±2.2</td>
<td>37.3±2.3</td>
</tr>
<tr>
<td>Hypogastric</td>
<td>11/12</td>
<td>&gt;52.8±4.0</td>
<td>&gt;54.6±4.0</td>
</tr>
<tr>
<td>Somatic</td>
<td>10/10</td>
<td>33.4±2.4</td>
<td>35.5±2.5</td>
</tr>
</tbody>
</table>

Data are means ± SE of the lowest Pves that induced urine leakage (LPP) and the maximal Pves without urine leakage below the LPP (MTP) in female rats. LPP and MTP were evaluated as the indexes of urethral resistance. Pelvic, hypogastric, or somatic nerves (to iliococcygeus/pubococcygeus muscles) were bilaterally transected. Leak/tested shows the number of rats with fluid leakage per tested rats in each group. Because the LPP and MTP values in sham-operated rats and hypogastric nerve-transected rats were not accurately evaluated given that values in rats with no leakage were included, no statistical comparison was performed.

To investigate which afferent and efferent nerves were responsible for the urethral resistance during stress condition, the LPP and MTP values were measured in spinalized rats whose nerves innervating the bladder, urethra, and pelvic floor muscles were bilaterally transected. To clarify the participation of the afferent information from the bladder, bilateral pelvic nerves were transected. All rats with the pelvic nerves transected showed fluid leakage form the urethral orifice, and the LPP and MTP values in this group were obviously lowered (by ~15 cmH2O) compared with those in sham-operated rats (Table 2). Since the LPP and MTP values in sham-operated rats were not accurately evaluated, because rats with no leakage were included, no statistical comparisons were performed.

To investigate the contribution of the urethral smooth muscle to the urethral closing pressure during stress events, the bilateral hypogastric nerves were cut. Almost all rats with hypogastric nerves cut showed fluid leakage (11 of 12 rats), while reductions in LPP and MTP were not observed (Table 2). To evaluate the contribution of striated muscles such as the external urethral sphincter muscle and the pelvic floor muscles, bilateral pudendal nerves and nerves to iliococcygeus/pubococcygeus muscles were transected. In all rats with somatic nerves transected, urinary incontinence was observed and the LPP and MTP values were reduced by >15 cmH2O compared with those in sham-operated rats (Table 2). In addition, the MTP value was quite close to the LPP value in each group, indicating that the rate at which the stimulus intensity was increased was appropriate for measuring the urethral resistance.

**Effects of duloxetine on reflex urethral closing responses.** To investigate whether duloxetine exerts its effects on the bladder-to-urethral reflexes, the effects on the reflex urethral closing responses induced by the increase in Pves were examined. In the spinal cord-transected rats, the abrupt elevation of Pves from 0 to 25 or 50 cmH2O induced reflex urethral closing responses in a Pves elevation-dependent manner (Fig. 3), as reported by Kam et al. (14). Ten minutes after intravenous injection of duloxetine (0.3 and 1 mg/kg iv), a significant
were 0.3 ml of saline. Before administration of the drug, the values of catheter were also examined when the bladder was filled with saline without momentary stress measured by the microtip transducer. Duloxetine increased the LPP and MTP values in a dose-dependent manner, whereas the vehicle did not. The increments induced by the drug were statistically significant compared with those in the vehicle-treated group (Fig. 5).

To examine whether the LPP-increasing effects of duloxetine are also active in spinal cord-intact rats, the LPP measurements were performed without spinal cord transection. Since the micturition reflex interferes with the evaluation of urethral resistance during urine storage phase in the spinal cord-intact rats, rats were anesthetized with halothane to abolish the micturition reflex. Ten minutes after intravenous injection of duloxetine, significant elevations of the LPP and MTP values were observed (LPP: $-2.1 \pm 1.6$ vs. $9.5 \pm 3.3$ cmH$_2$O, $P < 0.05$, Student’s $t$-test; MTP: $-1.6 \pm 1.7$ vs. $9.2 \pm 3.3$ cmH$_2$O, $P < 0.05$, Student’s $t$-test) at 0 and 1 mg/kg iv, respectively; $n = 5$), indicating that the LPP-increasing effects of this drug are also detectable under the spinal cord-intact condition with the use of electrical stimulation of abdominal muscles.

Effects of nerve transections on the duloxetine-induced increase in urethral resistance. To clarify the contribution of the bladder-spinal-urethral reflex to the increasing effects of duloxetine on the urethral resistance, effects of intravenous duloxetine at 1.0 mg/kg were examined in rats with nerves innervating the bladder, urethra, and pelvic floor muscles transected. In rats whose pelvic nerves were bilaterally transected, all rats ($n = 10$) showed fluid leakage (Table 1) at LPP values <60 cmH$_2$O. Duloxetine was intravenously administered in 5 of these 10 rats, and no increase in LPP or MTP values was observed (Fig. 6). In rats whose hypogastric nerves were bilaterally transected, 11 of 12 rats showed fluid leakage (Table 1) and 10 of 12 rats indicated LPP values <60 cmH$_2$O. Duloxetine was intravenously administered in 5 of these 10

Effects of nerve transection on the urethral resistance. To examine the effects of duloxetine on the urethral resistance, LPP and MTP values during electrical stimulation of abdominal muscles were compared before and 10 min after intravenous injection of this agent in spinal cord-transected rats. Duloxetine was administered in rats with LPP values <60 cmH$_2$O to evaluate the increasing effects of this drug. Twenty-two of 28 rats showed fluid leakage as mentioned above, and all of these rats showed LPP values <60 cmH$_2$O. Duloxetine at 0, 0.3, and 1.0 mg/kg was intravenously administered in 15 of 22 rats. Duloxetine increased the LPP and MTP values in a dose-dependent manner, whereas the vehicle did not. The increments induced by the drug were statistically significant compared with those in the vehicle-treated group (Fig. 5).

Fig. 3. Schematic illustrating the method of electrical stimulation (A) and typical recordings of the middle urethral responses measured by the microtip transducer catheter during increments of P$_{ves}$ (B). Responses are shown before (Pre) and 10 min after intravenous injection of duloxetine at 0.3 mg/kg iv. The chart shows the change in P$_{ves}$, and the middle and bottom charts indicate the middle urethral responses to elevation of P$_{ves}$ before and after the stimulating process, respectively.
rats, and no increase in LPP or MTP values was observed (Fig. 6). In rats whose somatic nerves (pudendal nerves and nerves to ilio coccygeus/pubococcygeus muscles) were bilaterally transected, all rats \((n = 10)\) showed fluid leakage (Table 1) at LPP values <60 cmH2O. Duloxetine was intravenously administered in 5 of these 10 rats, and no increase in LPP or MTP values was observed (Fig. 6).

**DISCUSSION**

Because SUI occurs when \(P_{ves}\) suddenly increases over the urethral resistance (4, 5, 10, 23), the method for evaluating the urethral resistance during an abrupt increase in abdominal pressure was developed in female spinalized rats. The electrical stimulation of abdominal muscles for 1 s induced sudden increases in intra-abdominal pressure and \(P_{ves}\) equally, and the increment of \(P_{ves}\) was almost lost when the abdomen was opened. Furthermore, the electrical stimulation also induced the elevation of urethral pressure, and this increment was completely lost when the abdomen was opened. These results suggest that the electrical stimulation induces the abdominal muscle contractions to increase intra-abdominal pressure, which in turn compresses the bladder, leading to elevation of \(P_{ves}\), and no direct electrical stimulation of the detrusor muscles, the urethral muscles, and the nerves innervating these organs contributes to the changes in \(P_{ves}\) and urethral pressure during stimulations. The degree of \(P_{ves}\) elevation depended on the stimulus intensities; conversely, the degree of \(P_{ves}\) elevation was easily regulated by changing the stimulus intensities, indicating that the measurement of the lowest \(P_{ves}\) value capable of inducing the fluid leakage (LPP) during momentary stress condition is possible. In addition, since the MTP and LPP values in each group were quite close, and the true urethral resistance should fall between these two numbers, evaluation of the MTP and LPP values should be sufficient to assess the urethral resistance.

In this study, the urethral resistance estimated by LPP and MTP was greatly reduced by bilateral transection of pelvic nerves in the spinal cord-transected female rats. It was also indicated in a previous study (14) that in the spinal cord-transected female rats, the elevation of \(P_{ves}\)-induced urethral closing responses are completely lost by cutting the bilateral pelvic nerves. Although the pelvic nerve contains both afferent and efferent fibers (9), the contribution of pelvic efferent fibers to the urethral resistance seems unlikely, at least in the spinal cord-transected female rats, because it also has been shown that the bilateral transection of both the hypogastric nerves and the somatic nerves (pudendal nerves and nerves to ilio coccygeus/pubococcygeus muscles) innervating the urethral muscles and pelvic floor muscles totally abolishes the \(P_{ves}\)-induced urethral contractions under pelvic nerve intact conditions (14). Thus the reduction of urethral resistance by pelvic nerve transection is most reasonably attributed to the ablation of afferent inputs from the bladder to the spinal cord.

Because duloxetine, a drug for SUI, has been suggested to increase the reflex urethral contractions by findings that it

---

**Table 3. Effects of duloxetine on middle urethral baseline tone in urethane-anesthetized female rats with bladder distension**

<table>
<thead>
<tr>
<th>Duloxetine, mg/kg iv</th>
<th>(P_{ves}), cmH2O Pre</th>
<th>Post</th>
<th>Change</th>
<th>Urethral Pressure, cmH2O Pre</th>
<th>Post</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Vehicle)</td>
<td>7.4±1.6</td>
<td>6.3±1.4</td>
<td>-1.1±0.5</td>
<td>19.9±4.2</td>
<td>22.4±3.6</td>
<td>2.5±1.5</td>
</tr>
<tr>
<td>1</td>
<td>7.8±2.2</td>
<td>5.7±1.4</td>
<td>-2.2±0.9</td>
<td>23.5±4.8</td>
<td>33.3±6.5</td>
<td>9.8±2.6*</td>
</tr>
</tbody>
</table>

Data are means ± SE of \(P_{ves}\) and urethral pressure before and after administration of duloxetine in 5 spinal rats whose whole bladder was distended with 0.3 ml of saline. *\(P < 0.05\) compared with the change after drug administration in vehicle-treated group (Student’s \(t\)-test).
increases the electrophysiological activity of external urethral sphincter muscles and augments the spinal reflex in cats (25, 26), we directly examined the effects of this drug on the reflex urethral closure in rats in the present study. Duloxetine increased the reflex urethral closing responses induced by elevating Pves, consistent with the previous idea in literature (25, 26) that this drug enlarges the reflex urethral closure mechanisms. Duloxetine also increased the urethral resistance measured by electrical stimulation of abdominal muscles in this study, and this effect was not observed in the pelvic nerve-transected rats, in which the reflex urethral closure was completely lost. Since treatment with the drug to enlarge the reflex urethral contractions caused the increase in urethral resistance, it is also suggested that the reflex urethral closure mechanisms are involved in the prevention of urine leakage during the abrupt increment of abdominal pressure. In addition, since the guarding reflex for urine storage is considered not to function during the voiding phase, duloxetine, which enlarges this reflex, is suggested to have excellent properties as a drug for SUI in that after drug treatment, the urethral resistance increases on demand (when abdominal pressure increase). Overall, from the findings of studies with nerve transection and those with duloxetine, it seems reasonable to conclude that the reflex urethral contractions induced by the Pves elevation contribute to the increase in urethral resistance during sudden elevation of abdominal pressure.

A variety of muscles, structures such as the smooth urethral sphincter muscle, the striated external urethral sphincter muscle, and the striated pelvic floor muscles, can exert their effects on urethral resistance (21, 25). In the present study, bilateral transection of the somatic nerves (pudendal nerves and nerves to iliococcygeus/pubococcygeus muscles) innervating the striated external urethral sphincter muscle and the striated pelvic floor muscles (2, 18, 20) clearly reduced LPP and MTP, whereas the transection of hypogastric nerves innervating the smooth urethral sphincter muscle showed less effect (increase in the percentage of rats showing urinary incontinence). These observations suggest that the contraction of striated muscle components maintains the urethral resistance during the stress condition induced by electrical stimulation of the abdominal muscle and that the involvement of the smooth muscle component is smaller than that of the striated muscle component. In the stress condition with sneezing, the urethra also contracts to maintain the urethral resistance, and cutting the somatic nerves totally abolishes the reflex urethral contractions during sneezing and greatly lowers the sneeze LPP, whereas the transection of hypogastric nerves has no effect on the reflex contractions (7, 15). From these findings, it seems reasonable to conclude that to prevent urinary leakage during the sudden increase in abdominal pressure within 1 s [electrical stimulation: 1 s in the present study; sneezing: <0.15 s (7, 15)], the functions of striated muscle component are very important. On the other hand, the reflex urethral contractions, which are induced by passive elevation of Pves, for 2 min by changing the height of the saline reservoir connected to the bladder catheter, are partially reduced by the bilateral transection of the somatic nerves or the hypogastric nerves, indicating that both the striated and smooth muscle components are involved in the urethral closing. Furthermore, the study of rat urethral functions using an ex vivo system for investigating the biomechanical properties shows that the smooth muscle component rather than the striated muscle component in the isolated urethra greatly contributes to the resistance to intraluminal pressure elevation (11, 12), although it is also known that the smooth muscle is not suitable to show the quick responses. Together, these findings indicate that it is highly conceivable that the contribution of urethral smooth muscle functions may be higher under the basal condition without stress or under stress conditions lasting for a long time.

Although the contribution of the smooth muscle component to the total urethral resistance during the momentary stress condition seems smaller than that of striated component without duloxetine treatment, the involvement of both the smooth and the striated muscle functions in the duloxetine-induced increment of the urethral resistance may be considered, because the transection of not only somatic nerves but also the hypogastric nerve alone greatly reduced the elevation of the LPP and MTP by duloxetine. Although this drug did not increase the middle urethral baseline tone when Pves was 0 cmH2O, it became elevated when the bladder was distended with 0.3 ml of saline. From these findings, it is conceivable that when Pves is 0 cmH2O, the guarding reflex for urinary storage via the bladder-splenic cord-urethra and the pelvic floor muscles is silent; however, when the bladder is filled with 0.3 ml of saline, the guarding reflex is active, and duloxetine enlarges this reflex to increase the basal urethral tone. With these findings, it seems reasonable to elucidate that the increase in the urethral resistance by duloxetine during the momentary elevation of abdominal pressure is caused, at least in part, by the elevated basal urethral tone before stress events. Because the middle urethral basal closure under the condition of bladder distension with 0.3 ml of saline was long lasting, and the hypogastric nerve-mediated peripheral mechanism of noradrenergic pathway for urinary continence is proposed (13), it is likely that the contribution of hypogastric nerve-mediated smooth muscle functions to maintain the basal urethral resistance is significant, and duloxetine, a 5-hydroxytryptamine and norepinephrine reuptake inhibitor, potentiates the noradrenergic pathway-mediated smooth muscle functions. Furthermore, since duloxetine is also suggested to increase the reflex contractions of the urethral striated sphincters via spinal mechanisms (25, 26), this drug may increase the cooperation of the smooth muscle-related basal closure and the striated muscle-mediated abrupt contractile closure during momentary increments of abdominal pressure. Further studies are necessary to clarify the details of mechanisms of duloxetine increasing the urethral resistance.

In summary, the urethral resistance during stress conditions within 1 s can be evaluated by measuring LPP and MTP during electrical stimulation of abdominal muscles in spinalized female rats. During momentary elevation of abdominal pressure, the guarding reflex via bladder-splenic cord-urethra has an important role in the elevation of urethral resistance to maintain the urinary continence.

**REFERENCES**